Abstract

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Title of diploma thesis: HMGB1 in bacterial infections

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Background: Detection of HMGB1 mRNA expression and HMGB1 at the protein level in blood and ileum during bacterial infection in gnotobiotic piglet as infection model. Evaluation of the course of infection with expression IL-8, IL10, TNF-α and TLR4 mRNA in ileum and IL-8, IL10 a TNF-α levels in blood and intestinal washes. Final assessment of HMGB1 as potential late marker of sepsis in pig.

Methods: 1) The groups of gnotobiotic piglets – germfree (control), infected (extracellular parasite *E.coli* O55 or intracellular parasite *Salmonella enterica* serogroup Typhimurium) and associated piglets (probiotic *E.coli* Nissle 1917), 2) determination of bacterial count as CFU in blood and intestine with cultivation on bacteriological media, 3) column-based total RNA isolation from intestine, 4) spectrophotometric RNA quantification and estimation of purity, 5) cDNA synthesis with combined primers (oligo d(T) and random n-mers), 6) real-time polymerase chain reaction with LNA probes, 7) real-time PCR data normalization to β-actin and relativization to mean value of gene transcription, 8) ELISA detection of HMGB1 and selected cytokines levels in blood plasma and intestine washes.

Results: 1) Activation of HMGB1 gene expression was the lowest in germ-free piglets infected with enteropathogenic *E.coli* O55, 2) the highest activation in most genes was found in case of *S*. Typhimurium – exception was IL-10 in *E.coli* O55, 3) the highest plasma levels of HMGB1 were in group infected with *E.coli* O55, 4) in both virulent bacteria HMGB1 level was elevated in intestine washes, whereas HMGB1 was not found in germ-free piglets and piglets associated with probiotic *E.coli*, 5) we found the highest plasma cytokine levels in case of *E.coli* O55, cytokine concentrations in intestine were elevated in both virulent bacteria, 6) mRNA expression for TLR4 was the most increased in piglets infected with *S*. Typhimurium.

Conclusions: Gene transcription of HMGB1 is constitutive. HMGB1 is present in plasma only in piglets under severe septic condition and levels corelate with expected mortality (*E.coli* O55 infection). Increased HMGB1 levels in intestine caused with virulent bacteria. Concentrations of selected reference cytokines are in accordance with recently published data and state of animal health. So it is possible regard HMGB1 as late marker of sepsis in pig as well as other animal species and human.